

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
15 August 2002 (15.08.2002)

PCT

(10) International Publication Number  
**WO 02/062329 A1**

(51) International Patent Classification<sup>7</sup>: **A61K 31/195**,  
31/19 // (A61K 31/195, 31:19)

(21) International Application Number: PCT/EP02/00640

(22) International Filing Date: 23 January 2002 (23.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
MI2001A000204 2 February 2001 (02.02.2001) IT

(71) Applicant (for all designated States except US): **HUNZA DI PISTOLESI ELVIRA E C. SAS** [IT/IT]; Via Gavirate, 7, I-20148 Milano (IT).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **PISTOLESI, Elvira** [IT/IT]; Via Gavirate, 7, I-20148 Milano (IT).

(74) Agents: **MINOJA, Fabrizio** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: THERAPEUTICAL AND NUTRITIONAL COMPOSITIONS CONTAINING PYRUVIC ACID AND GLUTAMINE, HAVING ANTIOXIDIZING ACTIVITY AND CAPABLE OF CONTROLLING OVERWEIGHT

(57) Abstract: Therapeutical and/or nutritional compositions, containing: A) pyruvic acid as such or salified with: I) minerals; II) basic amino acids; III) proteins or peptides containing basic amino acids; IV) nitrogen bases; and B) glutamine (and/or proteins and/or peptides containing it as such or salified).



**WO 02/062329 A1**

THERAPEUTICAL AND NUTRITIONAL COMPOSITIONS CONTAINING PYRUVIC ACID AND  
GLUTAMINE, HAVING ANTIOXIDIZING ACTIVITY AND CAPABLE OF CONTROLLING OVERWEIGHT

The present invention relates to therapeutical and nutritional compositions having antioxidizing activity and capable of controlling overweight, containing a mixture of two components.

More particularly, the present invention relates to compositions  
5 consisting of:

- 1) pyruvic acid or salts thereof,  
and
- 2) glutamine (and/or proteins, and/or peptides containing them).

Said compositions are an effective, well-tolerated nutritional  
10 supplement for increasing with surprising synergism the antioxidants  
defenses of the body, and for keeping under control overweight as well as  
any unbalances in the body fat mass (in excess) to lean mass ratio deriving  
from high caloric input of foods. All these beneficial results are always by  
far higher than the sum of the results obtainable by the separated  
15 administration of the same active nutrients.

The present invention therefore relates to pharmaceutical and/or  
dietetic compositions as well as to functional foodstuffs capable of  
preventing both excessive increase in the peroxidative processes and  
overweight, as it happens in aging and in many related pathologies (such as  
20 atherosclerosis, diabetes, hypertension, lipid dysmetabolies, tumors, etc.).

The compositions of the invention comprise two active principles  
represented by:

A) pyruvic acid or salts thereof with:

I) minerals such as Ca, Mg, K, Na, Zn, Cr, Va, and the like;

- II) basic and/or neutral amino acids capable of inducing an anorectic effect, such as arginine, histidine, asparagine, tryptophan, phenylalanine, tyrosine, leucine, and the like;
- III) proteins or peptides containing said basic and/or neutral amino acids;
- IV) nitrogen bases such as choline and derivatives, ethanolamine and derivatives, mono and dimethylethanolamine and derivatives, polyamines, and the like;
- and
- 10 B) glutamine (and/or proteins and/or peptides containing it as such or salified with lactic acid, pyruvic acid or other organic acids such as: fumaric, malic, oxalacetic, aspartic, citric, isocitric, acetic, propionic, butyric acids and the like.

In addition to the two above cited active principles, the compositions  
15 of the present invention can further contain other compounds conventionally used, such as:

- I) fibers and polysaccharides (such as inulins, pectins, celluloses, chitosans, maltodextrins, starches and flours, and the like);
- II) vitamins and mineral salts, single or in mixture thereof;
- 20 III) vegetable extracts both crude and as fractions enriched in their content in bioflavones, terpenes, tannins, polycosanols, and the like.

Examples of said vegetable extracts are:

- vegetable extracts having diuretic, fat metabolism stimulating and intestinal  
25 improving activities (for example extracts from artichoke, milk thistle (*Silybum marianum*), *Curcuma longa*, *Pierroriza kurroa*, etc.) and/or
- vegetable extracts having lymphokinetic and detoxifying activity (such as melilot (*Melilotus officinalis*), dandelion (*Taraxacum officinale*),

citrus essential oils, and the like); and/or

- vegetable extracts capable of activating fat lipolysis (such as coffee, tea, green tea, oolong tea, wax extracts of citrus, rice, sugar cane, and the like); and/or
- 5 - vegetable extracts capable of improving blood rheology, microcircle flow rate and NO release (such as Ginkgo biloba, Ginseng, and the like).

Pyruvic acid is present in amounts ranging from 0.1 to 2000 mg, preferably from 4 to 400 mg, per kg body weight of the treated subject.

10 Glutamine (and/or proteins and/or peptides containing it) is present in amounts ranging from 0.1 to 500 mg, preferably from 10 to 100 mg, per kg body weight of the treated subject.

Some examples of compositions of the invention are reported in the following.

Example 1:

- |    |                     |       |
|----|---------------------|-------|
| 15 | a) calcium pyruvate | 180 g |
|    | b) glutamine        | 70 g  |

Example 2:

- |    |                              |       |
|----|------------------------------|-------|
|    | a) calcium pyruvate          | 250 g |
|    | b) wheat flour oligopeptides |       |
| 20 | containing 29% glutamine     | 50 g  |

Example 3:

- |  |                              |       |
|--|------------------------------|-------|
|  | a) pyruvic acid              | 50 g  |
|  | b) wheat flour oligopeptides |       |
|  | containing 29% glutamine     | 200 g |

25 Example 4:

- |  |                     |       |
|--|---------------------|-------|
|  | a) calcium pyruvate | 90 g  |
|  | b) glutamine        | 133 g |
|  | c) inulin           | 777 g |

Example 5:

- |   |    |   |       |
|---|----|---|-------|
|   | a) | pyruvic acid  | 90 g  |
|   | b) | wheat flour oligopeptides containing<br>29% glutamine | 360 g |
| 5 | c) | oat fiber   | 500 g |

Example 6:

- |    |    |   |       |
|----|----|---|-------|
|    | a) | pyruvic acid or salts thereof                         | 90 g  |
|    | b) | wheat flour oligopeptides containing<br>29% glutamine | 360 g |
| 10 | c) | Betaine-chitosan                                      | 400 g |

Example 7: water-dispersible powder consisting of:

- |    |    |   |       |
|----|----|---|-------|
|    | a) | pyruvic acid  | 150 g |
|    | b) | L-glutamine and/or peptides containing it<br>(in glutamine)   | 360 g |
| 15 | c) | an amino acids mixture consisting of:<br>L-tryptophan (30 g), L-phenylalanine (150 g) and<br>L-arginine (150 g)   |       |
|    | d) | a vegetable extracts mixture consisting of:<br>green tea (300 g), artichoke (50 g), melilot (15 g), caffeine<br>20 extracted from tea and/or coffee (30 g), Ginkgo biloba (20 g),<br>polycosanols extracted from sugar cane (3 g), lemon<br>essential oils (5 g) and black pepper extracts<br>containing 1-piperoylpiperidine (1 g) |       |
| 25 | e) | a mixture of vitamins and mineral salts consisting of:<br>vitamin E (10 g), lipoic acid (8.5 g), vitamin F,<br>(linoleic acid and linolenic acid and/or highly<br>polyunsaturated derivatives thereof, 320 g), vitamin<br>B6 (1 g), folates (200 mg) and vitamin B12 (2 mg).  |       |

The various compositions described in the above examples can be used for the preparation of both functional foodstuffs (bread, pasta, crackers, biscuits or other bakery products; dressings, margarines, mayonnaise, sauces, beverages, fruit juices, soft drinks and the like; creams, chocolate, milk, cheese, yogurt, etc.) and galenic preparations consisting of vials, hard- or soft- gelatin capsules, tablets, sachets, effervescent tablets, chewing gums, syrups, etc.

### **Pharmacological and/or dietetic experimentation**

The pharmacological and/or dietetic characteristics of the compositions of the present invention were assessed by means of two series of tests on rat and humans, respectively.

In a first series of tests on rats, the animals were given a hypercaloric, hypertriglyceridemizing and hypercholesterolemizing diet. After twenty days of treatment, the following parameters were evaluated:

- 1) lipoperoxides levels in plasma, liver, brain and heart;
- 2) body weight changes;
- 3) cholesterol plasma levels;
- 4) triglycerids plasma levels.

50 Male rats, each weighing 150-200 g, were used. The animals were subdivided into 5 groups of 10 animals each:

**1<sup>st</sup> group:** control (C); 10 animals (control at time 0) received no treatment, 10 animals received for 20 days standard hypercaloric, hypertriglyceridemizing and hypercholesterolemizing diet consisting of:

20% casein; 3.5% mixture of oligoelements and mineral salts; 0.1% mixture of vitamins; 0.2% choline ditartrate; 2% cellulose; 0.5% cholesterol; 0.25% sodium cholate; 58.44% saccharose; 10.0% lard and 4.9% olive oil.

**2<sup>nd</sup> group:** animals treated with glutamine (G); animals received for 20

days the same diet as the controls except that 3.0 g of glutamine replaced in part a similar amount of casein (casein used: 17%).

**3<sup>rd</sup> group:** animals treated with pyruvic acid; animals received for 20 days the same diet as the controls except that 4.5 g of pyruvic acid replaced  
5 in part a similar amount of saccharose (saccharose used: 53.94%).

**4<sup>th</sup> group:** animals treated with glutamine + pyruvic acid; animals received for 20 days the same diet as the controls except that 3.0 g of glutamine and 4.5 g of pyruvic acid replaced respectively similar amounts of casein (casein used: 17%) and saccharose (saccharose used:  
10 53.94%).

The obtained results prove that the administration of the composition of the invention is capable of promoting, in rats subjected for 20 days to hypercaloric diet enriched in animal saturated fats and cholesterol, a surprising, synergistic effect in:

- 15       1) improving antioxidants defenses of plasma, liver, brain and heart;  
          2) reducing body overweight;  
          3) limiting excessive increase in plasma cholesterol and triglycerid levels, thereby promoting the accumulation of the lean body mass to the detriment of the fat body mass.

20       Said beneficial, therapeutical effects obtainable by the administration of the compositions of the invention are always significantly higher than the sum of the beneficial effects obtainable by administering separately the single components of the various compositions.

25       In a second series of tests in humans, the tested composition contained pyruvate (3.75 g/die) and glutamine (5 g/die) added with amino acids, vitamins, minerals and vegetable extracts in the same quali-quantitative composition as that reported in example 7.

The dietetic supplement, administered for 30 days, gave surprising beneficial effects in:

- a) significantly reducing overweight;
- b) significantly improving the lean mass/fat mass ratio;
- 5 c) significantly improving membrane fluidity of erythrocytes and lymphocytes;
- d) improving the antioxidant defenses of plasma lipoproteins.

Said therapeutical improvements obtainable by the administration of the composition as reported in example 7 were always significantly higher  
10 than those obtained when administering separately the different components of the above mentioned compositions, i.e.: a) pyruvate; b) glutamine; c) the fraction containing all of the other components of the mixture without pyruvate and glutamine.



## CLAIMS

1. Therapeutical and/or nutritional compositions, containing:
  - A) pyruvic acid as such or salified with minerals, basic amino acids,  
5 proteins or peptides containing basic amino acids or nitrogen bases;  
and
  - B) glutamine (and/or proteins and/or peptides containing it as such or salified).
2. Compositions as claimed in claim 1, wherein minerals are selected  
10 from: Ca, Mg, K, Na, Zn, Cr, Va.
3. Compositions as claimed in claim 1, wherein glutamine and/or proteins and/or peptides containing it are salified with lactic acid or other organic acids selected from the group consisting of pyruvic, fumaric, malic, oxalacetic, aspartic, citric, isocitric, acetic, propionic and butyric acids.
- 15 4. Compositions as claimed in claim 1, wherein pyruvic acid is present, as such or in salified form, in amounts ranging from 0.1 to 2000 mg, preferably from 4 to 400 mg, per kg body weight of the treated subject.
5. Compositions as claimed in claim 1, wherein glutamine is present in amounts ranging from 0.1 to 500 mg, preferably from 10 to 100 mg, per kg  
20 body weight of the treated subject.
6. Compositions as claimed in any one of the above claims, further containing fibers, polysaccharides, vitamins, mineral salts and vegetable extracts.
7. Compositions as claimed in claim 6, wherein fibers and  
25 polysaccharides are selected from the group consisting of inulins, pectins, celluloses, chitosans, maltodextrins, starches and flours.
8. Compositions as claimed in claim 6, wherein the vegetable extracts are selected from extracts of artichoke, milk thistle (*Silybun marianum*),

Curcuma longa, Pierroriza kurroa, melilot (*Melilotus officinalis*), dandelion (*Taraxacum officinale*), tea, green tea, oolong tea, coffee, Ginkgo biloba, Ginseng, rice, sugar cane, citrus fruits.

9. Foodstuffs containing the compositions of claims 1 to 8.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/00640

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/195 A61K31/19 //(A61K31/195,31:19)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, CHEM ABS Data, EMBASE, FSTA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 008 252 A (BEALE PAXTON K) 28 December 1999 (1999-12-28) abstract column 2, line 51 - line 61 column 3, line 58 - line 62 column 6, line 22	1-4, 9
X	US 5 889 040 A (BEALE PAXTON K ET AL) 30 March 1999 (1999-03-30) abstract column 2, line 23 - line 67 column 4, line 5 column 5, line 33 - line 40 example 1 column 9, line 1 - line 5 -/-	1, 2, 6-9

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

23 May 2002

Date of mailing of the international search report

10/06/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hornich, E

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 134 162 A (STANKO RONALD T) 28 July 1992 (1992-07-28) abstract column 1, line 10 - line 15 column 2, line 33 - line 50 column 4, paragraph 1 claims 1,3,4,7,8,10 ---	1,4,6,7, 9
X	MALAISSIE W J ET AL: "THE STIMULUS SECRETION COUPLING OF AMINO-ACID INDUCED INSULIN RELEASE METABOLIC INTERACTION OF L GLUTAMINE AND 2 KETO ISO CAPROATE IN PANCREATIC ISLETS" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 677, no. 1, 1981, pages 39-49, XP001079060 ISSN: 0006-3002 abstract ---	1
Y	US 5 215 750 A (KEANE II MICHAEL A) 1 June 1993 (1993-06-01) abstract column 2, line 37 - line 40 ---	1,6
Y	WO 99 02479 A (SUEDDEUTSCHE KALKSTICKSTOFF) 21 January 1999 (1999-01-21) abstract page 1, paragraph 2 page 6, paragraphs 3,4 ---	1,6
Y	EP 1 010 429 A (ROEDER 1956 FARMACEUTICI S P A) 21 June 2000 (2000-06-21) abstract column 2, line 39 - line 44 column 2, line 51 column 3, line 5 column 4, line 55 table 1 claims 1,2 ---	6
A	DE 199 35 306 A (SUEDDEUTSCHE KALKSTICKSTOFF) 29 June 2000 (2000-06-29) column 1, paragraph 2 ---	1
P,X	US 6 221 836 B1 (BEALE PAXTON K ET AL) 24 April 2001 (2001-04-24) abstract column 1, paragraph 1 column 2, line 65 - line 66 column 3, line 9 column 6, line 51 - line 58 column 7, line 38 - line 44 column 7, line 59 - column 8, line 3 example 1 ---	1-3,6-9
	---	-/--

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/00640

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	EP 1 097 646 A (AJINOMOTO KK) 9 May 2001 (2001-05-09) abstract claim 1 -----	1, 10

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-9 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to pyruvic acid and glutamine as such or salified.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 02/00640

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 6008252	A	28-12-1999	US 5756469 A	26-05-1998
			US 5716926 A	10-02-1998
			BR 9710585 A	24-10-2000
			CA 2261781 A1	05-02-1998
			CZ 9900255 A3	16-06-1999
			EP 0914108 A1	12-05-1999
			US 5919767 A	06-07-1999
			WO 9804253 A1	05-02-1998
			BR 9710586 A	21-03-2000
			CA 2261708 A1	05-02-1998
			CZ 9900249 A3	16-06-1999
			EP 0914109 A1	12-05-1999
			WO 9804254 A1	05-02-1998
			US 6221836 B1	24-04-2001
			US 5889040 A	30-03-1999
US 5889040	A	30-03-1999	US 5716926 A	10-02-1998
			US 6221836 B1	24-04-2001
			BR 9710586 A	21-03-2000
			CA 2261708 A1	05-02-1998
			CZ 9900249 A3	16-06-1999
			EP 0914109 A1	12-05-1999
			US 6008252 A	28-12-1999
			WO 9804254 A1	05-02-1998
US 5134162	A	28-07-1992	NONE	
US 5215750	A	01-06-1993	NONE	
WO 9902479	A	21-01-1999	DE 19729786 A1	14-01-1999
			AU 725505 B2	12-10-2000
			AU 8731298 A	08-02-1999
			BR 9810702 A	08-08-2000
			WO 9902479 A1	21-01-1999
			EP 0993433 A1	19-04-2000
			HU 0004638 A2	28-04-2001
			JP 2002507998 T	12-03-2002
			NO 20000122 A	10-01-2000
			NZ 501771 A	27-10-2000
			PL 337946 A1	11-09-2000
			SK 180799 A3	08-10-2001
			TR 200000018 T2	21-07-2000
			TW 404833 B	11-09-2000
			US 6342631 B1	29-01-2002
			US 5962734 A	05-10-1999
			ZA 9805908 A	23-02-1999
EP 1010429	A	21-06-2000	IT 1303578 B1	14-11-2000
			EP 1010429 A2	21-06-2000
DE 19935306	A	29-06-2000	DE 19935306 A1	29-06-2000
			GB 2345060 A	28-06-2000
			US 6232497 B1	15-05-2001
US 6221836	B1	24-04-2001	US 5889040 A	30-03-1999
			US 5716926 A	10-02-1998
			BR 9710586 A	21-03-2000
			CA 2261708 A1	05-02-1998

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 02/00640

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6221836	B1	CZ 9900249 A3	16-06-1999
		EP 0914109 A1	12-05-1999
		US 6008252 A	28-12-1999
		WO 9804254 A1	05-02-1998
EP 1097646	A	JP 2001128615 A	15-05-2001
		EP 1097646 A2	09-05-2001